CLAIMS

We claim:

1. A porous silk fibroin material comprising a three-dimensional silk fibroin body having interconnected pores, wherein the pores have a diameter of 10 to 1000 microns, wherein the material has a compressive modulus of at least 100 kPa.

2. The porous silk fibroin material of claim 1, wherein the pores have a diameter of 50 to 500 microns.

3. The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 150 kPa.

4. The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 200 kPa.

5. The porous silk fibroin material of claim 1, wherein the material has a compressive modulus of at least 250 kPa.

6. The porous silk fibroin material of claim 1, wherein the material has a porosity above 80%.

7. The porous silk fibroin material of claim 1, further comprising an additive.

8. The porous silk fibroin material of claim 7, wherein the additive is a biologically active or pharmaceutically active compound.

9. The porous silk fibroin material of claim 8, wherein the biologically active compound is a cell growth factor.

10. The porous silk fibroin material of claim 9, wherein the cell growth factor is a cytokine.

11. The porous silk fibroin material of claim 8, wherein the biologically active compound is a peptide that contains an integrin binding sequence.

12. A process for producing a porous silk fibroin material comprising the steps of:

    a) forming a silk fibroin solution comprising silk fibroin in an aqueous salt solution;

b) removing the salt and water from the fibroin solution to form a silk fibroin substance;

c) forming a polymer solution comprising about 5 to 35% by weight of the silk fibroin substance in a solvent selected from a group consisting of hexa-flouro-iso-propanol (HFIP), N-methyl morpholine N-oxide and calcium nitrate-methanol;

d) contacting the polymer solution of step c) with water-soluble non-toxic particles that are insoluble in organic solvents and have a diameter between about 50 and about 1000 microns;

e) placing said polymer solution into a form;

f) removing the solvent from the polymer;

g) contacting said polymer solution with an effective amount of β-sheet structure inducing agent to induce β-sheet structure and insolubility in aqueous solution;

h) leaching said polymer with a solvent in which said particles are soluble and polymer is insoluble to remove said particles from said polymer; and

i) drying said polymer to form a porous silk fibroin material.

13. The process of claim 12, wherein step d) comprises placing said polymer solution of step c) into a form containing water-soluble non-toxic particles that are insoluble in organic solvents and have a diameter between about 10 and about 1000 microns.

14. The process of claim 12, wherein in step f) said solvent is removed by sublimation or evaporation.

15. The process of claim 12, wherein the silk fibroin is derived from a silkworm.

16. The process of claim 15, wherein said silk worm is *Bombyx mori*.

17. The process of claim 12, wherein the silk fibroin is derived from a spider.

18. The process of claim 12, wherein the silk fibroin is genetically engineered silk.

19. The process of claim 12, wherein the β-sheet structure inducing agent is selected from the group consisting of methanol, 2-propanol, 1-butanol, isoamyl alcohol, and chloroform, and acetone.

20. The process of claim 12, wherein the aqueous salt solution comprises lithium bromide, lithium thiocyanate or calcium nitrate.

21. The process in claim 12, wherein the particles are selected from the group consisting of alkali metal and alkaline earth metal halides, phosphates and sulfates, sugar crystals, water-soluble polymer microspheres and protein microspheres.

22. The process of claim 21, wherein the particles are sodium chloride crystals.

23. The process of claim 12, wherein the polymer is leached with water.

24. The process of claim 12, comprising the step of adding an additive.

25. The process of claim 24, wherein the additive is a biologically active or pharmaceutically active compound.

26. The process of claim 25, wherein the biologically active compound is a cell growth factor.

27. The process of claim 26, wherein the cell growth factor is a cytokine.

28. The process of claim 25, wherein the biologically active compound is a peptide that contains an integrin binding sequence.

29. A porous silk fibroin material produced by the method of claim 12.

30. A porous silk fibroin material produced by the method of claim 12 and a biocompatible polymer.

31. The material of claim 30, wherein the biocompatible polymer is selected from the group consisting of polyethylene oxide (PEO), polyethylene glycol (PEG), collagen, fibronectin, keratin, polyaspartic acid, polylysine, alginate, chitosan, chitin, hyaluronic acid, pectin, polycaprolactone, polylactic acid, polyglycolic acid, polyhydroxyalkanoates, dextrans, polyanhydrides, polymer, PLA-PGA, polyanhydride, polyorthoester, polycaprolactone, polyfumarate, collagen, chitosan, alginate, hyaluronic acid and other biocompatible polymers.

32. A method for producing a tissue engineered construct comprising culturing mammalian cells on a porous silk fibroin material produced by the method of claim 12.

33. A method for producing cartilaginous tissue comprising culturing multipotent cells on a porous silk fibroin material of claim 1 under conditions appropriate for inducing cartilage formation.

34. The method of claim 33, wherein said conditions comprise nonessential amino acids, ascorbic acid-2-phosphate, dexamethasone, insulin, and TGF–β1.

35. The method of claim 34, wherein said nonessential amino acids are present at a concentration of 0.1 mM, said ascorbic acid-2-phosphate is present at a concentration of 50 ug/ml, said dexamethasone is present at a concentration of 10nM, said insulin is present at a concentration of 5 ug/ml and said TGF-β1 is present at a concentration of 5 ng/ml.

36. A method for producing cartilaginous tissue comprising seeding multipotent mammalian cells on a porous silk fibroin material produced by the method of claim 1 and implanting said material into a host.

37. A method for producing bone tissue comprising culturing multipotent cells on a porous silk fibroin material of claim 1 under conditions appropriate for inducing bone formation.

38. The method of claim 37, wherein said conditions comprise ascorbic acid-2-phosphate, dexamethasone, β-glycerolphoasphate and BMP-2.

39. The method of claim 38, wherein said ascorbic acid-2-phosphate is present at a concentration of 50 ug/ml, said dexamethasone is present at a concentration of 10nM, said β-glycerolphoasphate is present at a concentration of 7 mM and said BMP-2 is present at a concentration of 1 ug/ml.

40. A method for producing bone tissue comprising seeding multipotent cells on a porous silk fibroin material of claim 1 and implanting said material into a host.

41. The method of any of claims 33, 36, 37, 40, wherein the multipotent mammalian cells are selected from the group consisting of bone marrow stromal cells and adult or embryonic stem cells.

42. The method of claim 32, wherein said porous silk fibroin material has a 3-dimensional structure of a predetermined shape and said tissue forms the predetermined shape of said 3-dimensional structure.

43. The method of claim 33 or 36, wherein said porous silk fibroin material has a 3-dimensional structure of a predetermined shape and said cartilaginous tissue forms the predetermined shape of said 3-dimensional structure.

44. The method of claim 37 or 40, wherein said porous silk fibroin material has a 3-dimensional structure of a predetermined shape and said bone tissue forms the predetermined shape of said 3-dimensional structure.

45. The method of any of claims 33, 36, 37, 40, wherein said porous silk fibroin material further comprises an agent that enhances proliferation and differentiation of said multipotent mammalian cells.

46. The method of claim 45, wherein said agent is selected from the group consisting of bone morphogenic proteins, transforming growth factor β, dexamethosone, insulin, β-glycerolphosphate, epidermal growth factor, platelet derived growth factor, fibroblast growth factor, nerve growth factor, vascular endothelial derived growth factor, and insulin like growth factor.

47. The method of claim 32, wherein said mammalian cells are selected from the group consisting of hepatocytes, bile duct cells, islet cells, pancreatic cells, parathyroid cells, thyroid cells, gonadal cells, epithelial cells, nerve cells, heart muscle cells, skeletal muscle cells, blood vessel cells, lymphatic vessel cells, kidney cells, intestinal cells, chondrocytes, keratinocytes, fibroblasts, osteocytes, parenchymal cells, bone marrow cells, mesenchymal stem cells, embryonic stem cells, adult stem cells, endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells, and genetically engineered cells.

48. The method of any of claims 32, 33, 36, 37, 40, wherein said mammalian cells are autologous cells.

49. The method of claim 47, wherein said autologous cells are human cells.

50. The method of any of claims 32, 33, 36, 37, 40, wherein said cells are donor cells.

51. A 3-dimensional tissue produced by the method of claim 32.

52. A 3-dimensional cartilaginous tissue produced by the method of claim 33.

53. A 3-dimensional bone tissue produced by the method of claim 37.

54. A method for treating a subject suffering from tissue damage or loss comprising producing a tissue engineered construct of claim 32 and implanting said construct into the subject.

55. A method for treating a subject suffering from tissue damage or loss comprising producing a porous silk fibroin material by the method of claim 12 and implanting said material into said subject.

56. A method for treating a subject suffering from tissue damage or loss comprising producing the cartilaginous tissue of claim 33 and implanting said construct into a said subject.

57. A method for treating a subject suffering from tissue damage or loss comprising producing the cartilaginous tissue of claim.

58. A method for treating a subject suffering from tissue damage or loss comprising producing the bone tissue of claim 37 and implanting said construct into a said subject.

59. A method for treating a subject suffering from tissue damage or loss comprising producing the bone tissue of claim 40.